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Investigation to Characterize the Influence of Fixation Methods upon the Biomechancial Properties of Cadavers in an Impact Environment

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Abstract

This paper compares the biofidelity of fresh, frozen, and embalmed cadavers used for impact testing. For the past two years at the University of Virginia, cadavers have been preserved using experimental embalming methods and fluids. The original formulation of the preservation fluid was developed in Europe and has been used extensively for pedestrian impact studies at INRETS in France. Advantages of embalming over other preservation techniques include continuous access to the cadaver, virtual elimination of bloodborne infectious diseases, and the ability to provide sectional variation of tissue compliance. Sectional variation permits phenomena, such as muscle tensing, to be simulated in specified body regions while other areas of the body can remain unaffected. Sled, pendulum, and other impact apparatus provide dynamic comparisons of the thorax among the frozen, fixed, and fresh cadavers. In addition, mechanical properties of embalmed hard and soft tissues are investigated utilizing a static test machine. Preliminary results from these tests, combined with autopsy records and histology specimens, suggest both exceptional preservation and biofidelity of the embalmed cadavers.

Introduction

Throughout history, human remains have been preserved for religious ceremonies and educational study by a variety of methods. The practice of embalming truly began in ancient times with the Egyptians, who prepared bodies for resurrection. With the advent of modern warfare, soldiers killed in battle were often far from their native countries and preservation of the bodies for transport back to their homelands was necessary . The scholarly interests of the Renaissance spurned a fascination with medical science and resulted in the preparation of cadavers for anatomic study. Embalming in the modern era, however, is primarily for the benefit of funeral rituals. Modern embalming chemically treats the dead human body in order to reduce the presence and growth of microorganisms, to retard decomposition, and to restore an acceptable physical appearance [1]. Tissue preservation is accomplished through a chemical "fixation" or coagulation of the proteins within the tissue cells. The chemicals produce cross-linkages between adjacent proteins which are not normally present in the living tissue. The result is a complex latticework of inert, firm material that is no longer a suitable medium for bacteria. In addition, these inactive stabilized proteins are extremely resilient to the body cell's autolytic enzymes.

The degree and length of preservation depend on many variables such as the intrinsic body factors, extrinsic environmental factors, as well as the skill and equipment of the embalmer. The embalmer must assess all these factors and determine the appropriate concentrations of fluid and embalming methodology for each case. Arterial embalming involves the injection, distribution, diffusion, and drainage of a preserving solution through the arteries of the circulatory system. Arteries are chosen for embalming because, unlike the long veins, they do not possess a network of valves. After entering the arterial system, the fluid flows through the venous and capillary systems while simultaneously draining the body of blood and other tissue fluids. Through several active and passive transport processes, the solution passes from the capillaries to the extravascular tissue beds. It is the retained fluid, intravascularly and extravascularly, that works over time to embalm the body. At the Automobile Safety Laboratory (ASL), a multipoint or sectional injection

method is preferred. Embalming fluid is introduced into the circulatory system from primary injection sites shown in Bold print in Figure 1.

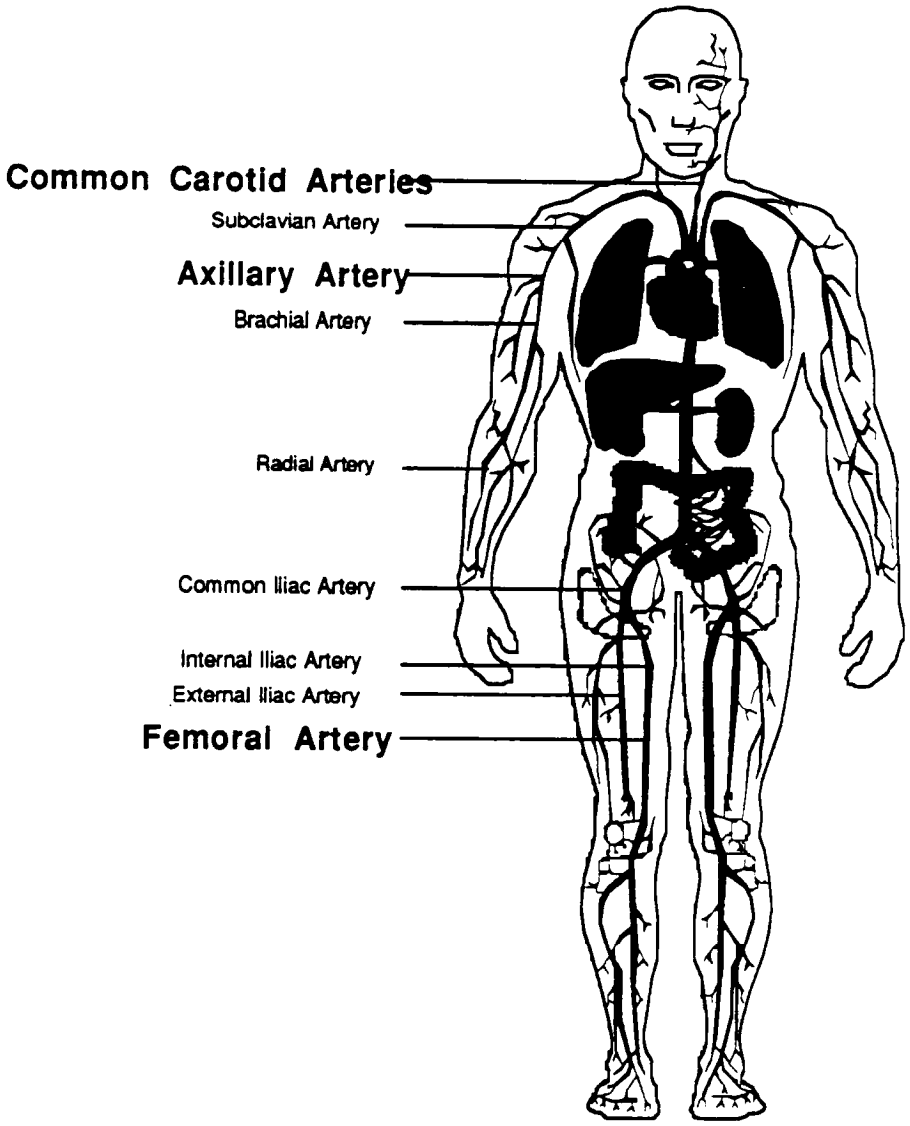

Figure 1. Primary and Secondary Injection Sites for Embalming

Primary injection sites indicate the locations used in conventional funeral home cadaver fixation. Secondary sites, shown in plain text, refer to locations where additional or supplemental embalming is possible, and may be used if inadequate distribution of the solution to a specific body

area occurs. Under certain circumstances, "overembalming" or excessive fixation of a body area may be desirable and its usefulness will be explored later in this paper. The abdominal cavity, however, is not embalmed separately. In funeral home embalming, the thoracic and abdominal cavities are embalmed separately by insertion of an extremely sharp instrument called a trocar. Injections through the cavity walls permit preservation in areas which are typically difficult to reach and also allow collected gas and fluid to be released . Damage is done to the internal organs, thus, the use of this method would render the subject ineligible for post-test injury analysis.

Winckler Fluid

In Europe, the Winckler fluid was developed in order to preserve cadavers for anatomical studies [2]. In refrigerated compartments, post-mortem storage intervals of several years have been reported. The Winckler fluid is an arterial embalming fluid, meaning that the fluid is introduced into the body through the arterial system. Drainage of blood and excess embalming fluid occurs through the venous system. The route of embalming fluid is similar to that followed by blood in the human body. Preservation by the Winckler method requires that an extremely slow flow rate and low pressure be maintained. The cadaver is embalmed in two stages over a twenty-four hour period. This technique allows for supersaturation of the body, resulting in equal parity of fluid in the tissue. Following fixation of the cadaver, the body is paced in a shroud pouch, which maintains high humidity levels, and stored in a 34°F to 37°F cooler.

The fluid is comprised of standard embalming ingredients but the quantities and embalming methodology is unique. A batch of the Winckler fluid contains the following:

Water (H_2O)	**9 L**
Phenol (C_6H_5OH)	**100 g**
Alcohol (C_2H_5OH)	**500 cc**
10% Formalin (HCOH)	**1500 cc**
Glycerine ($CH_2OHCHOHCH_2OH$)	**250 cc**
Chloral Hydrate ($C_2H_3Cl_3O_2$)	**500 g**
Sodium Sulfate (Na_2SO_4)	**125 g**
Magnesium Sulfate ($MgSO_4.7H_2O$)	**125 g**
Potassium Nitrate (KNO_3)	**250 g**

Each component plays a unique and vital role in the preservation of the body. Water constitutes the predominant solvent. Formalin, formaldehyde gas absorbed in water, preserves the body by altering the state of the tissue's proteins. It reacts with the amino acids to form an insoluble resin which is very resistant to enzymatic breakdown. Phenol, a very powerful disinfectant derived from coal-tar, controls bacterial, virucidal, and germicidal activity within the body. In addition, it assists formaldehyde in preserving the tissue. Phenol is a very hazardous chemical and extreme caution should be used when handling it. Alcohol chemically stabilizes the formaldehyde as well as acting as a moderately effective preservative. Glycerine is classified as a "humectant". Humectants increase the ability of embalmed tissues to retain moisture [1]. Glycerine also tends to be an excellent solvent for disinfecting chemicals. The exact role of chloral hydrate in the Winckler fluid has yet to be determined. It is probably used as a chemical stabilizer for formaldehyde, however, further experimentation with embalming fluids will determine its necessity. It is important to note that chloral hydrate is a sedative and is listed as controlled substance; acquisition requires a DEA registration number. Magnesium sulfate, sodium sulfate, and potassium nitrate are inorganic salts. These salts determine the osmotic properties of the

embalming solution, governing the rate at which the fluid is drawn from the capillaries into tissue spaces.

Histologic Investigation

To evaluate the efficts of preservation methods, tissue samples have been prepared from post-impact autopsies. Kidney and liver samples were removed from fresh, frozen, and embalmed cadavers. Frozen cadavers had been stored at -50°C and slowly thawed in a refrigerator during a one week period. All embalmed cadavers had been preserved with the Winckler fluid. The degree of autolysis, or cellular breakdown, was investigated for each sample. Loss of definition of the individual cells, loss of cellular relationships or overall tissue architecture, or changes as a result of post-mortem events characterized autolysis in the study.

Kidney sections were chosen because they are representative of an organ which is well preserved by the arterial embalming process. Kidneys produce urine by a filtration of the blood through a series of collection tubules. They are highly vasculated, relatively light in mass, not very dense and are thus well perfused by embalming solutions. The stage of autolyis within the tubules was used to characterize the degree of preservation of the kidneys.

Kidney samples from a typical autopsy are compared against frozen samples. The tubules from the tpical autopsy sample show only slight autolysis. The frozen sample demonstrates moderatly severe autolysis with sloughing, or shedding, of the epithelial cells. The epitheleal cells are the cells which line the outer walls of the tubules and tend to congregate in the center of the tubule with autolysis.

Kidney specimens are again shown in Figure 3. The embalmed cadaver 91-M-04 shows fairly extensive autolysis while the other embalmed cadaver 91-W-05 shows only slight breakdown of the tubular epithelium. Edema, or excessive fluid in the tissue, can be identified for the 91-W-05 subject, but the individual tubules are intact and well preserved. This discrepancy in degrees of

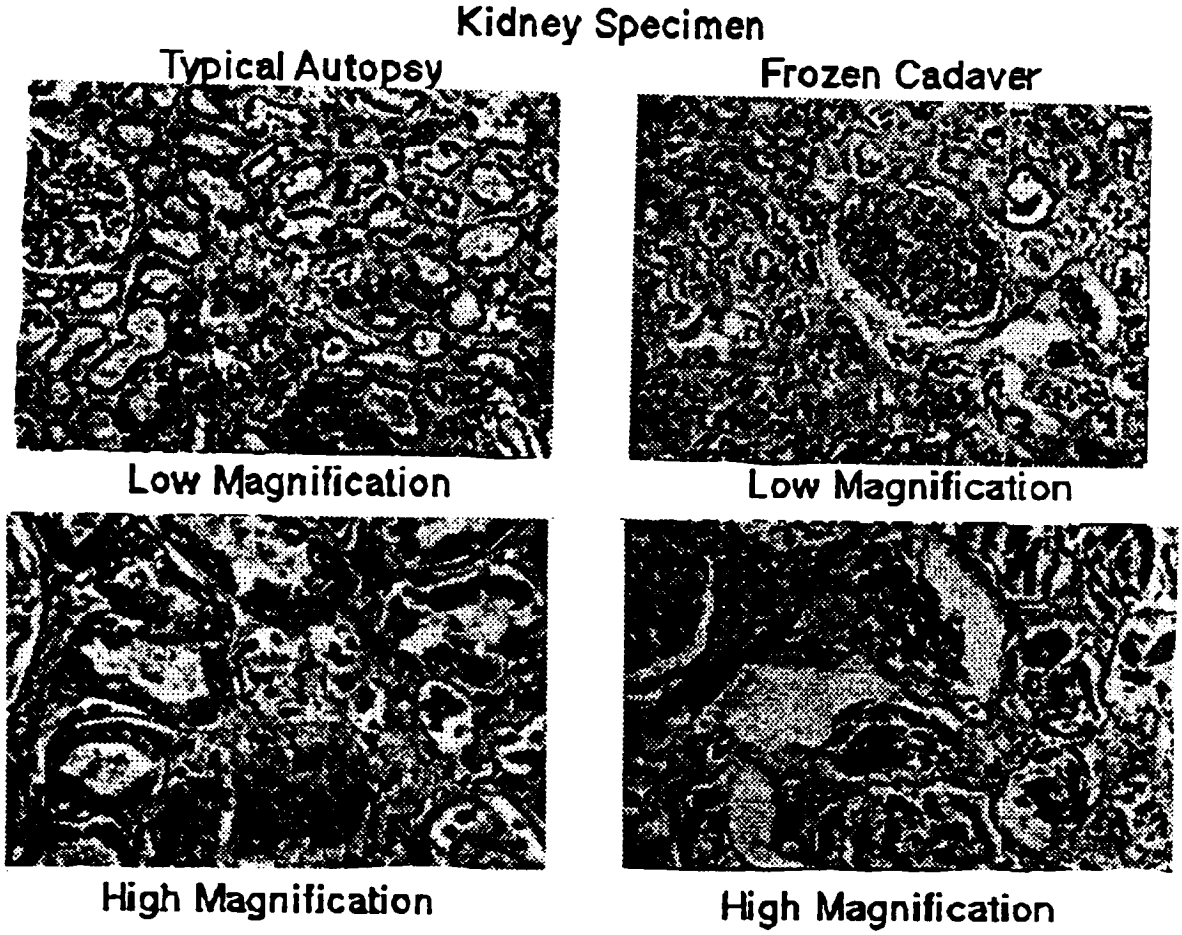

Figure 2. Kidney Specimens from Typical Autopsy (left) and Frozen Cadaver 91-M-01 (right)

preservation between the embalmed cadavers is explained by Table 1 showing subject characteristics. Since a constant amount of fluid had been used in the past, larger and heavier bodies were preserved to a lesser degree. The quantities of preservative found in the fluid were simply not sufficient to react with the body's proteins. In addition, earlier cadavers were

embalmed through only the femoral artery rather than the aforementioned multipoint method. Table 1 shows that subject 91-W-05 was multipoint embalmed and was of moderate weight (46.9 kg.). Subject 91-W-04, however, which weighed considerably more (74.6 kg.), was embalmed with the same quantity of Winckler fluid, and was injected through only a single point.

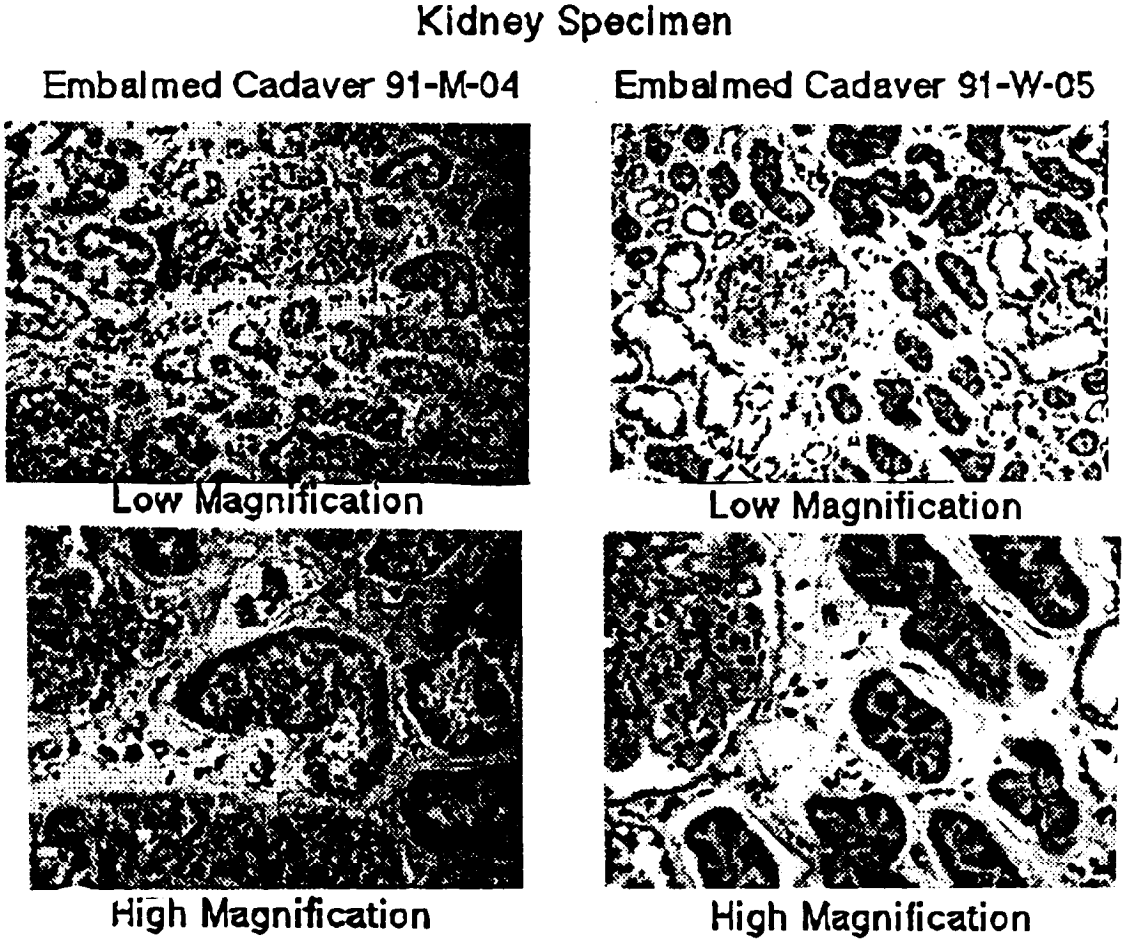

Figure 3. Kidney Sections from Embalmed Cadaver 91-M-04 (left) and Embalmed Cadaver 91-W-05 (right)

Unlike the kidneys, the liver is preserved to a much lesser degree through embalming. It is very difficult to preserve by arterial embalming because of its large mass and high density. Cells within the liver are organized into cordlike structures which form bile drainage ducts. This cord structure as well as the cells which comprise it are used to estimate the level of autolyis.

Once again, figure 4 demonstrates the differences that can occur within embalmed cadavers. As evident from the photographs, the liver sections on the left (Cadaver 91-W-05) represent a very slight degree of autolysis while the sample on the right (Cadaver 91-M-04) exhibits moderately severe autloysis. The left samples show the liver cords still intact while the right sections show a distinct disruption of the cords of the liver cells. Similar to the preservation of the kidneys, the livers exhibit greater autolysis for heavier bodies that are embalmed through only a single point.

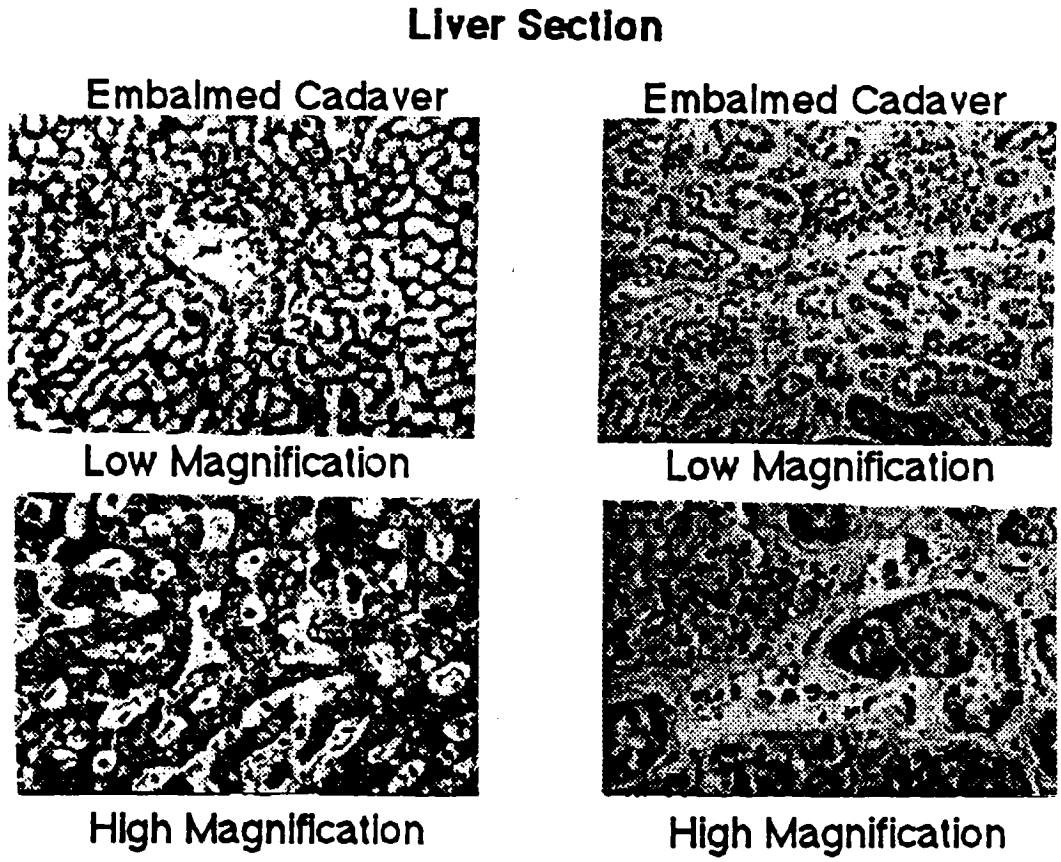

Figure 4. Liver Sections from Cadaver 91-W-05 (right) and Cadaver 91-M-04 (left)

To optimize the tissue preservation, a case analysis of the subject should be performed to determine the amount of embalming fluid and the associated pressures and flow rates. A rule of thumb for the amount of embalming fluid needed is one gallon for every 40 to 50 pounds of body

weight. It is recommended, however, that extra fluid always be prepared, since many unforeseen factors can affect the required amount and rate of fixation. Case histories of each subject allow the embalmer to analyze these factors and to modify the procedures and concentrations of fluid. Alcoholics, for example, have tissue which is very difficult to fix due to long term abuse of their body and organs; a moderate or strong solution in ample quantities should be utilized. Obesity poses many problems for the embalmer. For obese subjects, one gallon of embalming fluid should be prepared for every 25 to 30 pounds of weight. This increase in the required amount of solution is necessary because the lipid material does not absorb the embalming fluid and interstitial pooling results. Arteriosclerosis, commonly associated with obesity, makes drainage difficult and may require higher injection pressures. Purge, defined as the evacuation of a substance from the external orifice of a body, is also a problem with obese subjects [1]. The head should be elevated with a rubber block to prevent purge during the embalming process. Diabetes causes poor peripheral circulation and often results in dehydration and poor firming of the tissues. Ruptured aneurysms, defined as localized dilations of an artery, prevent proper distribution of the arterial fluid [1]. Congestive heart failure, another common cause of death, can result in the coagulation and congestion of blood in the heart. Extreme changes in the cells and corresponding tissues are the result of patients subjected to chemotherapy. Drugs used in chemotherapy can inactivate the embalming fluid by causing the buildup of nitrogenous wastes or decreasing the permeability of the cell membrane [1].

Table 1 illustrates the considerations necessary to evaluate the amount and strengths of embalming fluid needed for the cadavers tested at the UVA Auto Safety Lab. The aforementioned problems with heavy cadavers, constant amounts of embalming fluids, single site injection, and the freezing of cadavers are summarized.

Proposed Testing

The validation of the UVA embalming technique for cadavers in an impact environment will be explained using two approaches. Dynamic and Static testing of the body components, such as hard and soft tissue, will be conducted. In addition, tests performed on the cadavers will provide a better understanding of the aggregate interaction of hard and soft embalmed tissue.

Table 1. Subject Data

Subject	Age	Weight (kg)	Cause of Death	Post-Mortem Interval (Days)	Preservation Technique	Histology Evaluation
91-M-01	75	98.2	Bacterial Infection	252	Frozen (-50°C)	Moderate Autolysis
91-W-03	63	60.3	Cardiac Arrest	273	Frozen (-50°C)	Moderate Autolysis
91-M-04	75	74.6	Abdominal Aneurysm	64	1 Point Embalmed	Moderately Severe Autolysis
91-W-05	53	46.9	Cardiac Arrest, MI	117	2 Point Embalmed	Slight Autolysis
91-W-06	53	79.9	Cardiac Arrest, MI	115	2 Point Embalmed	Severe Autolysis
91-M-07	65	65.2	MI, Coronary Artery Disease	161	2 Point Embalmed	Minimal Autolysis
Typical Autopsy	74	-	Diabetes Hypertension	2	Fresh	-

In the past, extensive testing on the mechanical properties of fresh, frozen, and embalmed tissue samples has been performed. Unfortunately, the results have often inconclusive and inconsistent. An exploration of previous research and the associated findings is presented below.

Frozen Tissue Testing

Initial testing of fresh versus frozen tissue samples was performed by Frankel in the early sixties [3]. He noticed no difference in the breaking strengths of fresh and frozen human femurs. Concurrently, Nachemson investigated the effects of refrigeration and freezing on lumbar intradiscal pressures [4]. He concluded that no statistically significant differences in hydrostatic pressures within intervertebral discs of cortical bone could be discerned between fresh and frozen (-25°C) specimens. In 1965, Sedlin prepared small specimens from cortical samples which he believed were indicative of the mechanical behavior of the whole bones [5]. Comparing the mechanical properties of fresh and frozen bones stored at -20°C for 21 to 28 days, human femoral samples were subjected to tensile and bend tests. He found no marked differences. Rahn et al. employed a Vicker's hardness test to characterize the elastic and viscoelastic properties of fresh rabbit tibias and those frozen at -20°C for 7 days [6]. Once again, the bones from the two specimen groups exhibited no dissimilitudes. In 1984, Pelker et al. investigated the effects of various freezing methods on the biomechanical properties of rat bone [7]. Freezing of specimens at temperatures of -20°C , -70°C, and -196°C did not adversely affect the strength of long bones tested in torsion or of vertebral bones tested in compression. Later, Lee and Pelker characterized shear fractures of proximal rabbit femurs by comparing fresh and frozen (-20°C for one to two weeks) samples [8]. Histologic sections demonstrated no significant differences in either the patterns of shear load or stress to failure between the fresh and frozen/thawed specimens.

In 1971, Menz analyzed the structural changes that occur in muscle, nerves, and leucocytes as a result of the freezing and thawing process [9]. He discovered that rapid freezing to low cryogenic temperatures (-196°C) caused small (less than one micron) intracellular ice cavities to form. Tissue architecture and cellular relationships were maintained. Conversely, less rapid freezing at higher temperatures (-15°C to -60°C) was characterized by the growth of large ice cavities which completely disrupted the tissue structure. More recently, Ralis investigated the effects of sub-zero temperature storage and subsequent thawing of dissected muscles, tendons, and spines [10]. Freezing caused a noticeable shortening of the fibers which led to the development of

deformities resulting from the unopposed shortening of fibers. After thawing , fibers were very easily elongated and joints showed an increased range of motion.

Panjabi et al. (1985) explored the changes in the biomechanical properties of cadaveric spinal units that occur after the freezing and thawing process [11]. Specimens were tested at various intervals (1 day to 232 days) as well as various temperatures of freezer storage. It was found that long-term storage did not alter the physical properties of the spinal specimens. The spinal units, because of their diversity of components (bone, ligaments, cartilage, etc.) , were thought to be representative of the musculoskeletal structure of the body.

In summary, the previous research indicates that the freezing and thawing process, does not appear to adversely affect hard tissue at any storage temperature or interval. The effects of freezing and thawing on soft-tissue, however, are not clear: Menz and Ralis noted changes in frozen tissue that would invalidate the use of freezing for tissue storage, however, Panjabi tested spinal units with soft tissue components and noticed no differences. More research is needed.

Embalmed Tissue Testing

Carothers et al. pioneered research in the effects of formalin on hard tissue. Conducting bend tests, they determined that embalming with formalin significantly increased the bending strength of human long bones [12]. In 1951, Carothers, Calabrasi, and Smith investigated the effects of embalming on the compressive strength of human compact bone specimens [13]. They concluded that a loss of compressive strength of approximately thirteen percent, occurred when the embalmed bone was tested. McElhaney et al., in the mid-sixties, extensively researched the mechanism by which embalming affected the mechanical properties of bone [14]. Tensile and compressive specimens were tested in both the fresh and embalmed states using four embalming solutions. These solutions were comprised of varying amounts of alcohol, phenol, glycerine, formalin, and water. McElhaney measured and compared physical properties such as the modulus of elasticity , the ultimate strength, the maximum strains to failure, and the Rockwell hardnesses.

Results varied among the various embalming solutions but some properties, such as a loss of compressive strength and an insignificant increase in hardness, seemed to remain constant.

In the same year, Evans conducted tensile tests on human tibial samples [15]. He noticed substantial increases in the tensile strength of embalmed specimens. While conducting research on the mechanical properties of bone, Evans' findings showed increases in tensile and hardness properties and a corresponding decrease in the ultimate compressive strengths. It is interesting to

Table 2. Mechanical Bone Tests Performed by McElhaney and Evans

MECHANICAL TESTING OF BONE

	McElhaney (1964) Fresh	McElhaney (1964) Embalmed	% Change	Evans (1973) Fresh	Evans (1973) Embalmed	% Change
Ultimate Tensile Strength (psi)	12,400	12,300	-0.8	12,289	13,513	+10
Max. Tensile Strain (in./in. $X10^{-6}$)	4,420	4,400	-0.5			
Modulus of Elasticity in Tension (psi $x\ 10^{+6}$)	3.00	2.90	-3.3	2.14	2.49	+16
Ultimate Compressive Strength (psi)	22,200	19,480	-13.5	27,171	23,289	-14
Max. Comp. Strain (in./in. $X10^{-6}$)	5,450	4,840	-11.2			
Modulus of Elasticity in Compression (psi $x\ 10^{+6}$)	4.10	4.00	-2.4			
Rockwell Hardness	95.0 (H)	97.0 (H)	+2.1	-3.1	11.4	+

compare McElhaney's and Evan's results which are often conflicting. Even though McElhaney choose beef femurs and Evans chose adult human tibias , the table 2 reveals quite disparate results for tissue samples that have been identically preserved with equal amounts (33%) of alcohol, glycerine, and formalin.

It is important to note the glaring discrepancies, such as in the modulus of elasticity, which occur; Evans found significant increases of 16%, while McElhaney found decreases of -3.3%. McElhaney's decreased modulus, though not statistically significant, is at least suggestive of a downward trend.

Performing similar tests, Sedlin, like Evans, noticed an increase in the modulus of elasticity of human femurs in 10% formalin [5]. In the late sixties and early seventies, Yamada and Evans discovered uniform increases of approximately ten percent in the mechanical properties when comparing all varieties of embalmed and fresh bone [16]. These incongruities warrant further research.

As with hard tissue testing, investigators have arrived at conflicting results for the testing of soft tissues treated with formaldehyde. Fundamental experiments performed by Highberger, who researched the structural stability of collagen fiber aggregates, determined the influence of formaldehyde in the leather tannery process [17]. He concluded that formalin treated connective tissue showed tensile strength decreases of up to 65% when compared with fresh fibers. In a follow-up investigation, Compton tanned fiber bundles from kangaroo tail tendons and his results, like Highbergers', demonstrated significant tensile strength reductions with formaldehyde treatment [18]. Independently, Roddy and Mao processed whole pieces of steer leg tendons and found no change in the dry strength of collagen fiber aggregates [19]. Since their results were counter to earlier findings, Gustavson performed an exhaustive study of the procedural differences between Roddy and Mao and all other investigators [20]. Roddy and Mao treated whole pieces of tissue and then "teased" individual fibers from these gross pieces of tissue. In contrast, all other researchers had initially isolated single fibers and then proceeded to fix them with formaldehyde. According to Gustavson, interchain crosslinking, produced by tanning agents, does not affect the cohesive forces between the wider gaps between the molecular chains in the gross pieces studies. Therefore, single fiber experiments are more effectively tanned than the gross pieces and are consequently subjected to "case hardening". Verifying Gustavson's hypothesis, Mendoza and Milch tested gross pieces of embalmed goatskin and, as anticipated, found no changes in the tensile

properties of the tissue [21]. Stucke researched the rupture patterns of fresh and formaldehyde fixed tendons. In his experiments, final ruptures of fresh human Achilles tendons resulted from successive partial tears while fixed Achilles tendons ruptured much more suddenly and uniformly [22]. In addition, the stress-strain curves of the treated samples were much more uniform than the fresh. Viidik and Lewin investigated four modes of gross piece tissue storage and the affect each had on the mechanical properties of tissue [23]. The results of their soft connective tissue tests are summarized in table 3.

Addition signs indicate significant increases in the values of the properties that occurred

Table 3. Effects of Storage Methods on Properties of Connective Tissue
Viidik and Lewin (1966)

	Failure Energy	Failure Load	Elongation at Failure	Ruptures
Saline (5 h.)	+	+	+	+
Saline (24 h.)				+
Frozen (-20°C)	+			+
10% Formalin		-		

relative to tissues in the fresh state, while subtraction symbols signify substantial decreases. It is extremely interesting to note that saline, which is commonly used for storage of biological material, significantly affected the material properties of connective tissue.

Cadaver Testing

Since the late sixties, limited testing has been performed on embalmed cadavers. Due to the tissue rigidity and the joint inflexibility associated with cadavers embalmed for anatomical studies, it was commonly accepted that embalmed cadavers lacked the biofidelity required for impact studies. Several researchers performed basic comparison tests on the thoraxes of embalmed and

unembalmed cadavers which, when combined with recent results at UVA, indicate that embalmed cadavers may be preferable for impact testing.

Patrick et al. performed static and dynamic thoracic stiffness tests on embalmed cadavers [24]. Static tests used a chain fall to lower 4" channel suspended from a spring scale onto the chest. An alternative approach utilized a hydraulic test machine to apply the loads. Static, human volunteer tests also provided a reference for comparative purposes. The volunteers pulled themselves against a 6" diameter padded target and deflections were photographically recorded. Dynamic forces were applied during sled tests via a 6" diameter impactor outfitted with a load cell. Deflection time-histories were recorded for all subjects on high speed film by monitoring a rod and flag apparatus that protruded from the back of the subject.

Nahum et al. compared the thoracic stiffnesses of four embalmed and seven unembalmed cadavers under static and dynamic loads [25]. The data from earlier testing of three embalmed cadavers was included for comparison purposes. A great deal of variance was noted between the two groups of cadavers; which was associated with a variation in embalming techniques and the length and method of storage. In addition, it is interesting to note that the post-mortem interval before embalming varied from 2 to 18 days. Obviously, considerable discrepancies due to tissue breakdown were to be expected.

Nahum limited static test loads to 140 pounds in order to prevent damage which could impair dynamic tests. Dynamic loads were applied via a 42.5 pound impactor at the midsternal level of the thorax. The 6" striker mass was outfitted with a triaxial load cell and accelerometer to record force and acceleration time histories. Deflections were measured by one of two methods: either an electromagnetic coil or a combination of a steel rod deflectometer and high speed camera. Using these methods, the researchers determined that some degree of damage could be expected for deflections on the order of 1.5 to 2 inches. However, the average age of the subjects was nearly 67 years old and the deflection threshold should be considered with regard to this fact. As anticipated, the thoracic stiffnesses of the embalmed subjects proved to be significantly greater than that of the unembalmed cadavers.

Stalnaker et al. performed static tests on both cadavers and human volunteers [26]. In addition, ten cadavers were impacted with a six-inch diameter rigid cylinder weighing 22 lbs. Impact velocities averaged 13 mph and forces were measured with an attached load cell. Using photometric techniques, deflections were measured for all subjects. Static tests were performed on an Instron Test Machine at a rate of 2.0 inches per minute The impacting surface was a six-inch

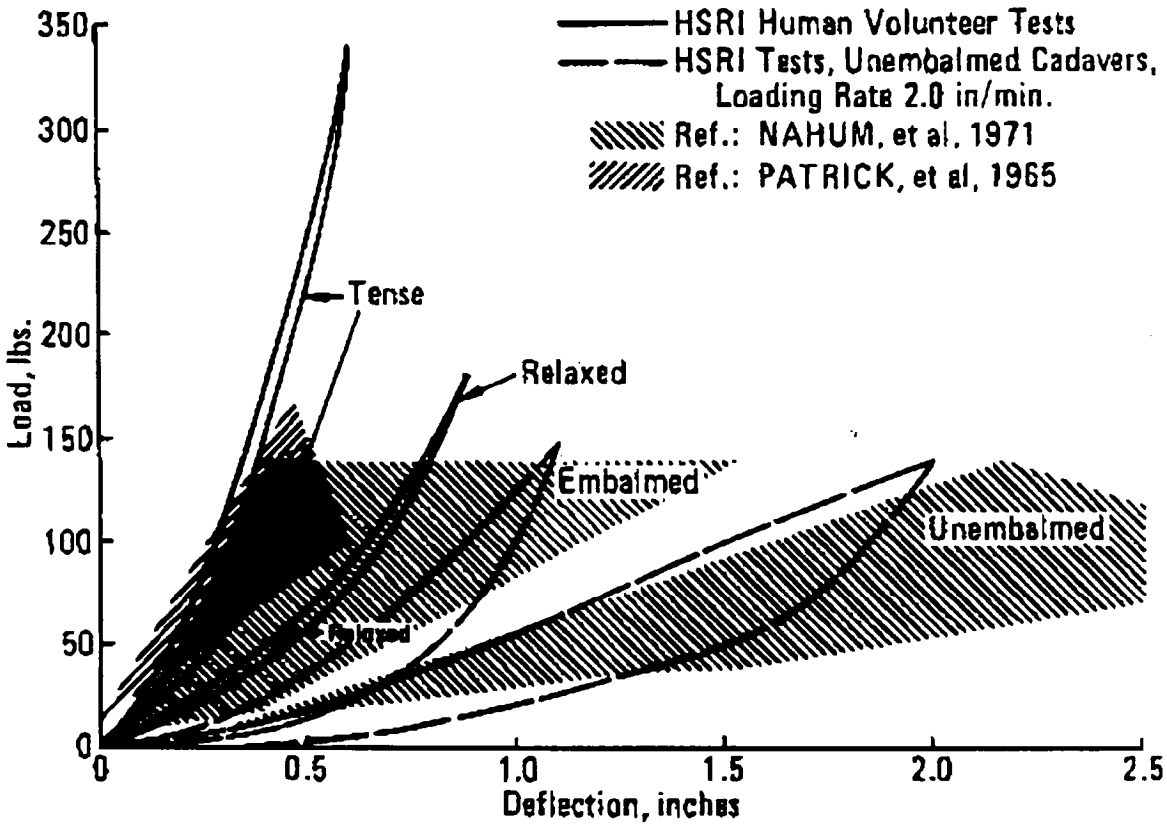

Figure 5. Cadaver and Human Volunteer Thoracic Static Tests

metal disc affixed to the crosshead of the Instron. In order to interpret the effects of muscle tensing on force and deflection time histories, initial static tests were performed on the volunteers with an alternate apparatus. The results from static tests performed by Stalnaker, Patrick, and Nahum are shown in figure 5.

The embalmed cadavers were found to be significantly stiffer than the fresh unembalmed subjects. Perhaps more interesting to note, however, is that the human volunteers in the relaxed state were found to have a stiffness comparable to that of the embalmed cadavers tested by Patrick. In addition, the diagram indicates that tensed volunteers have significantly higher stiffnesses than either the embalmed subjects or relaxed volunteers. Recalling the higher stiffness values associated with Nahum's three cadavers used for comparison purposes, stiffnesses of this magnitude should be obtainable in cadavers by adjusting the duration of storage and the method of embalming. Joint flexibility, however, must not be sacrificed for the associated increases in tissue stiffness.

Proposed Research

Thoracic and abdominal displacements during static loading will be investigated with the aid of a static test machine. Conventionally embalmed, Winckler embalmed, frozen, and fresh cadavers will be studied. Force-deflection curves will be used to characterize the stiffness of the thorax using chestbands as the primary recording devices, but machine crosshead travel, and photographic documentation will provide supplemental measurements. Deformation of the entire chest region during crushes from single point loads, circular plates, channel bars, and steering wheel rims will be monitored. Embalmed cadavers will be initially tested in the unembalmed state and then again after embalming. The static tests will be used to tailor local stiffnesses in the simulation of muscle tensing. Ideally, static stiffnesses would be predetermined for a particular body region and local fixation would be repeatedly performed until the desired tissue rigidity is achieved. These tests will provide a baseline of thoraco-abdominal behavior for dynamic studies and also begin to characterize the mechanical changes that occur in the embalming process. All static loads will be limited to non-injury levels and the subject's suitability for dynamic testing will be radiographically verified.

The static test machine will be utilized to study mechanical properties of biological materials. Three and four point bend tests will be done on fresh, frozen, and embalmed human ribs. In addition, compressive and tensile femur specimens will be cut and machined to exact

tolerances for testing. The load cell, strain gages, and extensometers will measure breaking loads, ultimate strengths, and ultimate elongations or contractions. Stress-strain curves will be generated and the elastic moduli will be calculated. Creep limits, rupture patterns, and fracture mechanics of the specimens may also be investigated. All properties will be compared among the different test groups as well as against previous research.

Uniaxial testing of soft tissue specimens from the frozen, fresh, and embalmed cadavers will be performed on the static machine. Specimens taken before and after preservation should allow rates of decomposition and the associated strength and mechanical properties of the tissue to be characterized. Hall Effect Transducers will measure strains while loads will be monitored by the test machine's load cell. Rate sensitivity will be investigated to the extent that the test machine permits. This work will complement an joint histologic investigation currently being done by the UVA Auto Safety Lab and the UVA Pathology Department.

Using a commercially available stress device, ligament range of motion and overall joint flexibilities will be measured before and after preservation. Lateral pressures will be applied to one side of the joint, which will be braced inferiorly and superiorly on the opposite side. Adjusting the pressures, displacements will be measured with lateral radiographs.

An impact pendulum has been designed and constructed for the dynamic testing of cadavers. The relatively small mass of the impactor permits nondestructive testing to be performed. Any given location of the thorax can be impacted repeatedly without causing damage to the outer tissue or the underlying infrastructure. Subjects will be fully instrumented with tri-axial accelerometers on the sternum, ribs, thoracic vertebrae, and pelvis. Up to three chestbands, two on the thorax and one on the abdomen, will be placed on the cadaver. Pulmonary and arterial pressurization will simulate average *in vivo* pressures, which will be continuously monitored by miniature pressure transducers in the descending aorta and the aortic arch. Force-deflection curves, and transfer functions if necessary, will be generated for all mount locations. Statistical auto-correlations and cross-correlations will be calculated for the cadaver and impactor acceleration time histories. Chest band contours should exhibit behavior consistent with the side, overhead,

and oblique high speed photography. Cadavers will be tested initially in the fresh state and compared to later tests performed in the frozen and embalmed states.

An impact table, which will utilize a shoulder belt as the primary load path, will be designed and constructed. Preliminary testing will be performed on an impact table on loan from Transport Canada. Impact energy levels will be limited so that data can be compared with existing dummy and human volunteer results from tests performed by Transport Canada (Biokinetics) and INRETS. Interaction with the seat belt will be monitored by chest bands and LVDTs while the belt load will be recorded by force transducers. Once again, nondestructive loads and velocities will be used to allow for pre-fixation and post-fixation comparisons.

Finally, sled tests have been performed with fresh, embalmed, frozen cadavers. Since the majority of the UVA subjects will be embalmed, cadaver data will be compared with fresh cadaver sled tests from Heidelberg and with frozen cadaver sled tests from the Medical College of Wisconsin. Subjects have been instrumented with chestbands and arrays of accelerometer at some or all of the following locations: upper spine, lower spine, fourth ribs, eighth ribs, upper sternum, lower sternum, and pelvis. Cadavers will be compared on chestband contours and measured accelerations, velocities, and deformations as well as injury criteria.

Conclusion

Evident from the histologic sections previously shown, preservation of embalmed cadavers can be exceptional. Using the Winckler fluid and multi-site embalming, decomposition of the tissue can be virtually halted for time periods up to one year. In addition, many of the mechanical properties of soft and hard tissue are unaffected by the embalming process. In some cases, those properties that are affected can be advantageously altered to more closely simulate living humans.

Today, the ubiquitous presence of blood-borne infectious diseases should be of concern to all researchers using unembalmed and frozen cadavers. Embalming fluid contains many powerful disinfectants which eradicate many of these diseases. In addition, drainage during embalming removes blood and associated pathogens from the body reducing their concentrations.

Continual access to the embalmed cadavers is a distinct advantage over other storage techniques. Subjects can be instrumented over several weeks and the mount of instrumentation is only limited by the number of data acquistion channels available. In addition, testing over several days can be performed and scheduled. If mechanical problems with the sled or test apparatus occur, the embalmed cadaver can simply be restored in the cooler until the problems are remedied.

The approximation of muscle tensing should be obtainable by varying the rate of embalming and the composition of the preservation fluid. Local stiffnesses, such as neck or thoracic stiffnesses, could be produced by ligating the required blood vessels and firming the tissue by continuous injection. Through experimentation, stiffness setpoints and the corresponding strength and amount of fluid would be determined.

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DISCUSSION

PAPER: **Investigation to Characterize the Influence of Fixation Methods upon the Biomechanical Properties of Cadavers in an Impact Environment**

SPEAKER: Jeff Crandall

QUESTION: John Ryan, Wayne State

About how much food do you use in that Winkler process? How much do you put in each cadaver?

A: As you saw the main ingredient was water which is 9 liters but the total comes to just a little over 10.5 liters.

Q: It has been my experience with embalmed cadavers that joints tend to get very stiff, not always, but occasionally we get one that you can't even bend.

A: I believe that's because they've been using a bad embalming technique or bodies used for anatomical studies. That may be because you're using either a stronger formalin or a 30% formalin, we're using a 10% formalin, or perhaps you're using a larger amount of formalin. We have not found, upon exercising the cadaver, an excessive tearing of tendons or ligaments or anything like that upon exercising the joints.

Q: We have found in some cases, rarely, but in some cases the legs are so stiff that you can't bend them at the knees without breaking them.

A: That is not the case at all with our cadavers. In fact, if you take a fresh or frozen cadaver that's been thawed and line it up against one of our embalmed cadavers, I don't think you could actually see a difference in the tissue upon palpation.

Q: Approximately what ages-have you got a correlation between (cadaver) ages and stiffnesses?

A: No, I don't. As I said, most of this testing here is proposed testing; this is what we propose to do and at this time we have a limited range of it. Our average age is probably somewhere in the mid- to upper 50's I would say.

Q: Jeff Pike,Ford Motor Co.

Just a suggestion for the next phase of your very interesting study, because the cadavers tend to be older, not only in your study but in most studies and the human volunteers tend to be on the young side, it might be real worthwhile if you have access to include some younger cadavers, younger at the time of death, in the next phase of your

study. And also, I noticed that most of the cadavers in the study died of MI and that sort of compounds the aging affect; if you do use the younger cadavers it might be especially interesting if you could screen for those that didn't die of coronary disease and see what sort of effect it has on the profusion as well.

A: That's interesting and I wish we could get younger cadavers. We're pretty limited in the availability. But thank you.

Q: Guy Nusholtz, Chrysler

This is sort of in response in part to John's question about the stiffness of the joints. Traditionally, when we had an embalmed cadaver, you stick these needles in and blow them up with god-awful pressure to ensure that you got the fluid everywhere you had to get it. Then the cadaver could just go hang on the rack for a month and let the fluid drain down and then wash it off of the floor. In this case, what's being done here is much more selective and the risk here is not, although there is a risk of some stiffening of the joints, the risk here is more towards not getting enough fluid in so that you get all of the organs fully embalmed.

A: Along the lines of that, something I did forget to mention which perhaps I should have is we don't use the standard embalming done by a funeral home or someone like that, taking about 2 hours. We do use a super saturation of the tissue over a 24 hour period, use a very low pressure, comparable to a gravity embalming technique. This is a very slow, very low pressure.

Q: When you go to control your stiffness, how are you planning to do it? Are you planning to selectively inject and then test the tissue and see if it meets some criteria and if it doesn't meet, it's too wimpy, does that mean you inject some more? How do you take care of it?

A: That is a possibility because you can always inject more fluid, in other words, if we go and we do a static test on a leg or something and find the stiffness isn't coming out in the static range in which we desire, we can always go and add more of the mixture or just add more formalin. We can always go and make it stiffer but we cannot take it away, unfortunately.

Q: Mike Walsh, Hartley Associates

In your selective stiffness my question is, are you planning on or have you done any injection into the specific muscle groups that you're interested in? What we did a number of years ago at CALSPAN was we were trying to simulate some neck stiffness, we were injecting neck muscles with formalin and it seemed to be working very well although we didn't do the engineering analysis that you've done on it. So do you plan on doing this for your selective stiffness?

A: Yes, we will do some engineering analysis. What I can tell you at this point is actually we made a mistake once and made up the wrong fluid and embalmed the legs with a different fluid than what we embalmed the torso and the head. And what you saw was a marked difference. You saw a great deal of an increase in the stiffness. We didn't quantify it, we just noted it and that's all we've done to this point.